FIELD TRIALS OF THE SUNTAN PROMOTING EFFECTS OF METHOXSALEN*

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From the beginning of the enthusiasm for the use of furocoumarin compounds to promote tanning in the treatment of vitiligo, or in promoting tolerance in persons who tolerate actinic rays poorly, the testimonial evidence of enthusiastic users suggested that we were dealing with an extremely potent tanning agent. Tanning through this discussion, refers to melanin pigmentation and not to tanning in the chemical sense of alteration of proteins such as used in the preparation of leather.

In 1955 an extensive study on 50 test subjects in the Arizona State Prison was reported by Fitzpatrick *et al.* (1). In this study a criss-cross design of exposures to sunlight following the oral administration of 50 mg. of methoxsalen was carried out which showed multiple effects of the methoxsalen combined with sunlight. These changes included erythema, edema and pigmentation. The pigmentation was still evident a year later when some of the same men were used in another study. On the basis of the 1955 study at the Arizona State Prison it was concluded that methoxsalen did not provide a primary protective effect but potentiated several of the skin's responses to ultraviolet light. Other clinical evidence, as shown in the color photographs, involved volunteers given 30 mg. of methoxsalen, or placebo, two hours prior to solar exposures. Area 124 in Figure a represents active drug plus one hour of exposure on days one, three and five. Area 105 was similarly exposed on days two, four and six, but after ingestion of placebo. The photograph was taken on the twelfth day. In Figure b the same procedure was employed in a subject with less capacity for pigmentation. In the face of these dramatic potentiated responses and continued vivid testimonial evidence a study was carried out in the spring of 1956, at the Arizona State Prison with refined statistical design, an unfortunate amount of lay press publicity, and smaller doses of methoxsalen.

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FIELD TRIAL AT THE ARIZONA STATE PRISON, 1956

A. *Experimental design.* The experiment consisted of two parts. In the first part twenty subjects were given various doses of methoxsalen and identically appearing placebos in a criss-cross design in which each subject received a series of exposures on the left side of his back on the first day, and a series of exposures on the right side of his back on the second day of the test period. The design of the test exposure windows is indicated in Fig. 1a. The hatched areas indicate the areas exposed to sunlight on a vertically compressed scale. Eight windows were exposed on the left side and eight on the right side of the back. Four windows were exposed in sequence to provide sequential 20 minute exposures and three windows were exposed to provide sequential 40 minute exposures to the Arizona sun near solar noon. A final window was exposed for a total of 80 minutes. The left side of the back was exposed on March 18, 1956, and the right side on March 19, 1956. Following these exposures the subjects received no further exposure to sunlight. Visual grading and reflectance readings with the Photovolt Model 610-T reflectance meter were carried out at intervals after the exposure. The pattern on the skin is indicated in Fig. 1b.

In this design an attempt was made to counterbalance the effects of days at 0 *vs.* 20, 0 *vs.* 40 and 20 *vs.* 40 milligram dosage. In addition, subjects with the same dosage on right and left side were included as a check on meteorologic variation and on possible drug carryover from the first day and the possible effects of physio-

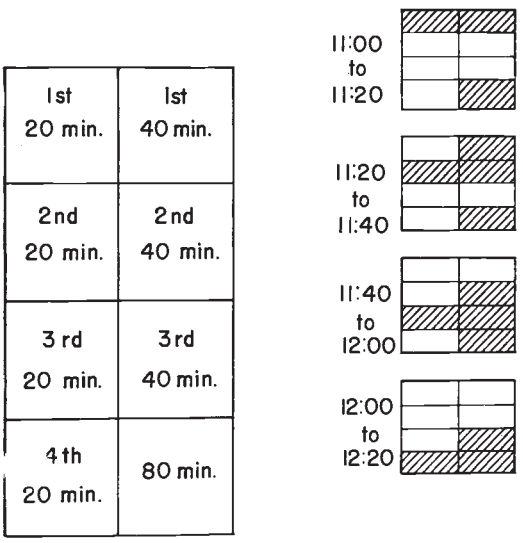

FIG. 1a

logical carryover from the effects of the initial exposure.

The second study at Arizona in the spring of 1956 was an attempt to demonstrate the protective action of repeated doses of sunshine with methoxsalen. To accomplish this, ten white male prisoners were exposed on the right side and left side of the back alternately for six consecutive days. Three of them received 20 mg. of methoxsalen two hours prior to exposure on the left side; three others received 20 mg. of drug prior to exposure on the right side. Two subjects received 20 mg. of methoxsalen prior to exposure on both the left and right sides and two subjects received placebo capsules two hours prior to exposure on both sides. On the first day, exposures were made on the left side. Subsequent exposures were as follows: second day right, third left, fourth right, fifth left, and sixth right. Twenty, forty and sixty minute windows were exposed on each side of the back.

It was hoped in this design that the repetitive exposures and the criss-cross design would to a large extent remove the effect of variations in solar radiation intensity between the different days. Two days following the last exposure under the described conditions, the 20, 40 and 60 minute windows were crossed with an exposed strip for two hours preceding solar noon in an attempt to demonstrate the presumed protective effect of the previously administered sunlight and methoxsalen. The general pattern is diagrammed in Fig. 2a. and depicted photographically in Fig. 2b.

B. *Results of the studies at the Arizona State Prison, 1956.* The results of the 20-man study with various doses of methoxsalen and various durations of solar exposure provided more information on the erythema-promoting effects of methoxsalen than on the pigment-producing effects. This resulted in part from the relatively short period of follow-up and in part from the fact that most of our reflectance meter data at that time were obtained with the blue, amber and green tristimulus filters, and did not include red filter readings (2). The results are indicated in Figures 3 and 4. In these figures the mean values for reflectance difference from adjacent unexposed skin have been plotted in three-dimensional form on isometric graph paper. The sample sizes are indicated in the diagrams, and it should be noted that each subject provided only two of the dosage levels, that is, some subjects received 20 and 40 mg. of methoxsalen, some received 0 and 40 mg., and others 0 and 20 milligrams. In addition there were some "weather" controls who received identical placebo or drug doses. All of these have been averaged to indicate the relationship between duration of solar exposure and the number of milligrams of methoxsalen ingested two hours prior to solar exposure. Figure 3 depicts this relationship as measured twenty hours after exposure, at which time the response was almost entirely erythema. Figure 4 shows the relationship as measured one week after exposure when tan was the principal effect. These graphs suggest that in part of the range tested a milligram of oral methoxsalen is about equivalent to a minute of Arizona sunshine at solar noon during the month of March, in promoting response of the skin to sunlight.

Experiments of this type are extremely vulnerable to differences in the ultraviolet variations produced by slight variations in weather conditions, as particularly emphasized by the findings that one of the sequential 40-minute windows showed a decreased response in all subjects although no change in insolation was evident during the experiment.

The experiment in which 10 men were exposed

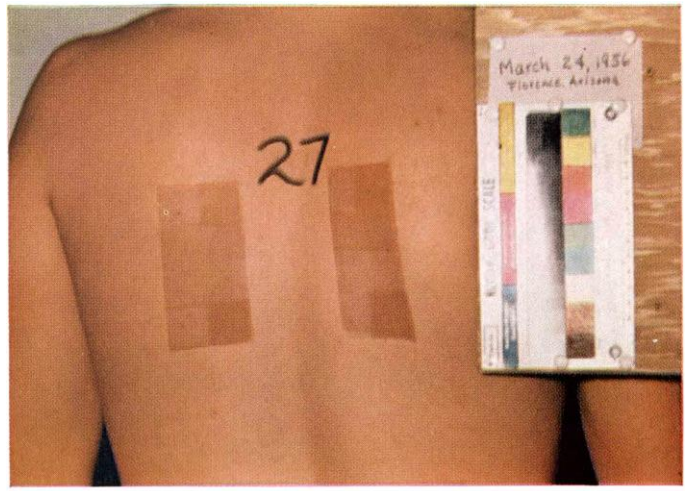

Figure 1b

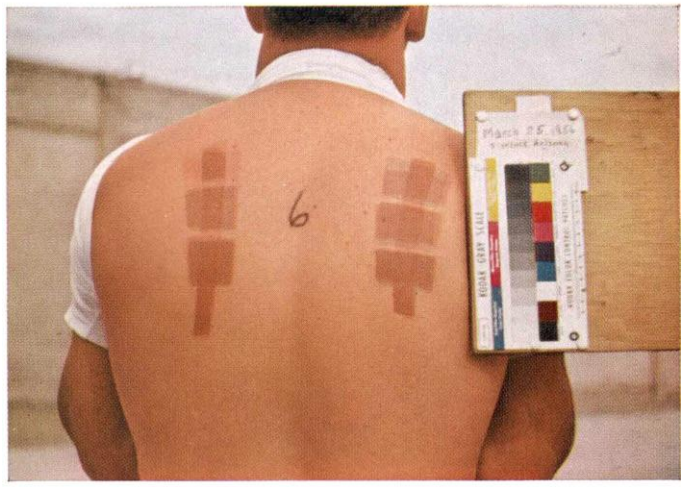

Figure 2b

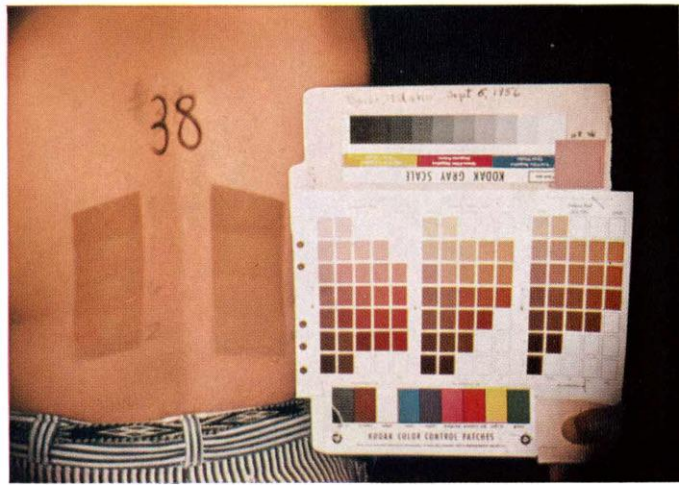

Figure 8

for 20, 40, and 60 minutes for 6 days with 20 mg. of methoxsalen criss-crossed with placebo failed to show a difference between the placebo and psoralen sides. The two hour solar challenge produced identical erythema and tanning reactions on the methoxsalen and placebo sides. It was not demonstrated that 20 mg. of methoxsalen administered with solar exposures on alternate days provided any more protection than similar exposures without methoxsalen.

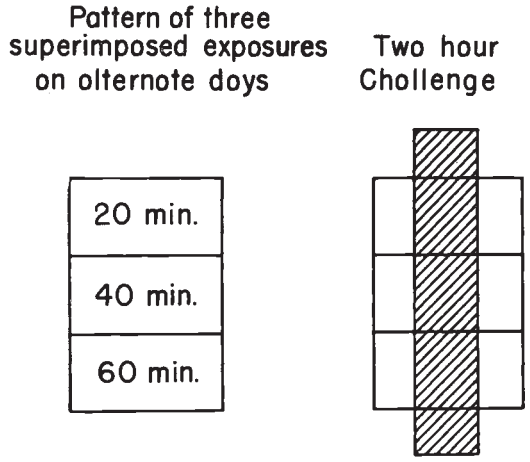

FIG. 2a

STUDIES DONE AT THE IDAHO STATE PRISON IN BOISE DURING THE SUMMER OF 1956

A. *Experimental design.* Among the possible explanations for the failure to produce the uniform evidence for tanning effects of methoxsalen in the 1956 Arizona experiments were the following:

1. The time scale of the experiments was too compressed.

2. There might be sufficient drug carryover so that drug administration one day would have some effect the next day. Therefore, a criss-cross design based on alternating days would produce potentiation on both sides and obscure drug effects. This seemed a reasonable hypothesis although it did not find definite support on inter-individual comparison of the few suitable cases in the Arizona test subject data.

To meet these major criticisms of the earlier experiment, we chose for study 30 prisoners at the Idaho State Prison who had not had any exposure on their backs for a period of at least 6 months. Ten men were exposed at a time in a rotating schedule. This type of schedule was organized not only to confound the effects of weather variations into the experimental design but also because of the mechanical difficulties in handling a large experiment with one investi-

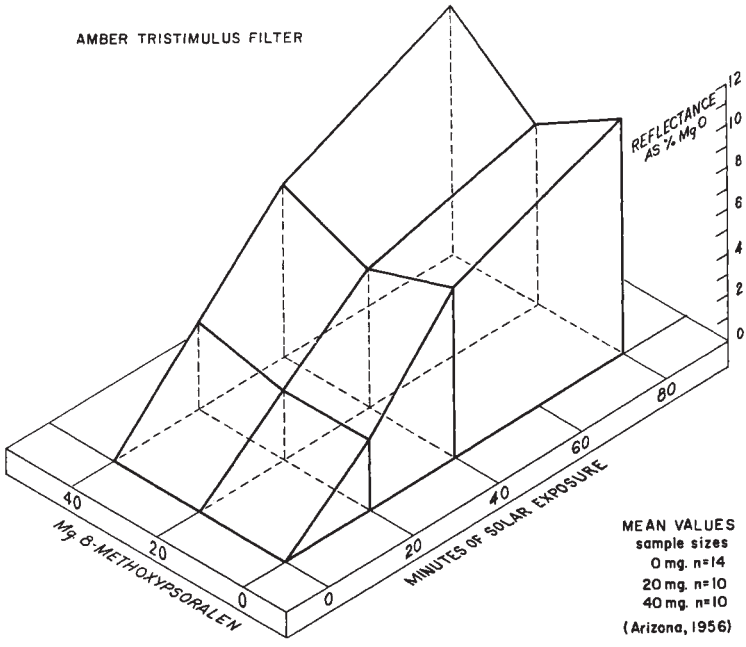

FIG. 3

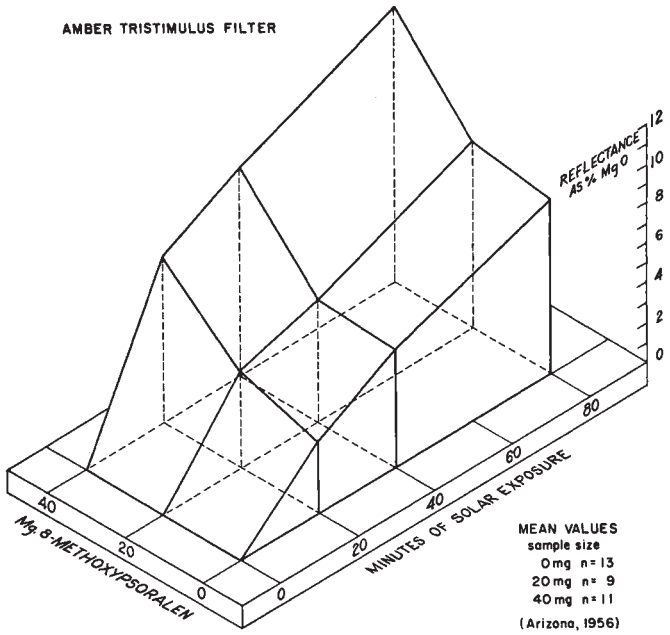

FIG. 4

Exposure time in minutes

First 3 exposures	Exposure 4	Exposure 5	Exposure 6
40	60	80	100
	40	40	40
20	30	40	50
	20	20	20

FIG. 5

gator (J.D.I.). The subjects received alternate exposures with placebo and 20 mg. of methoxsalen in a criss-cross randomized design. The exposures were so spaced that a week occurred between drug or placebo exposures and therefore a three to four day period between drug and placebo exposures ruled out or minimized the possible effect of carryover of the drug. By extending the interval between solar exposures and by continuing over a longer period of time another risk was assumed in the experiment: that of possible change in the spectral composition of sunlight between the end of June and the beginning of September.

In this study the solar exposures were made in the prison yard around solar noon as in the Arizona studies. Unexposed areas of the back were masked with oil cloth held in place with adhesive tape. The adhesive tape served as a sharp margin to the exposure windows.

B. *Results of the Idaho 1956 experiments.* The first three weeks (three exposures placebo, three exposures 20 mg. methoxsalen) did not produce significant differences between drug and placebo. The experiment was therefore continued with the additional feature of progressive increase in exposure time as indicated in Figure 5. Three further exposures were made.

There gradually emerged a small but statistically significant difference between control and placebo sides. For reasons which have not yet been ascertained correct identifications on visual gradings were made more frequently when methoxsalen was given with exposure on the right side. In the "best" visual grading the methoxsalen side was correctly identified 17 times, incorrectly identified once, and 12 subjects were rated as ties.

During this study, in anticipation of accurately quantifying the effects, about 22,000 visual readings were made of erythema, pigment and trauma. At the same time about 8,000 reflectance meter readings were made with a red glass filter and with tristimulus blue, green and amber filters. The total number of measurements on

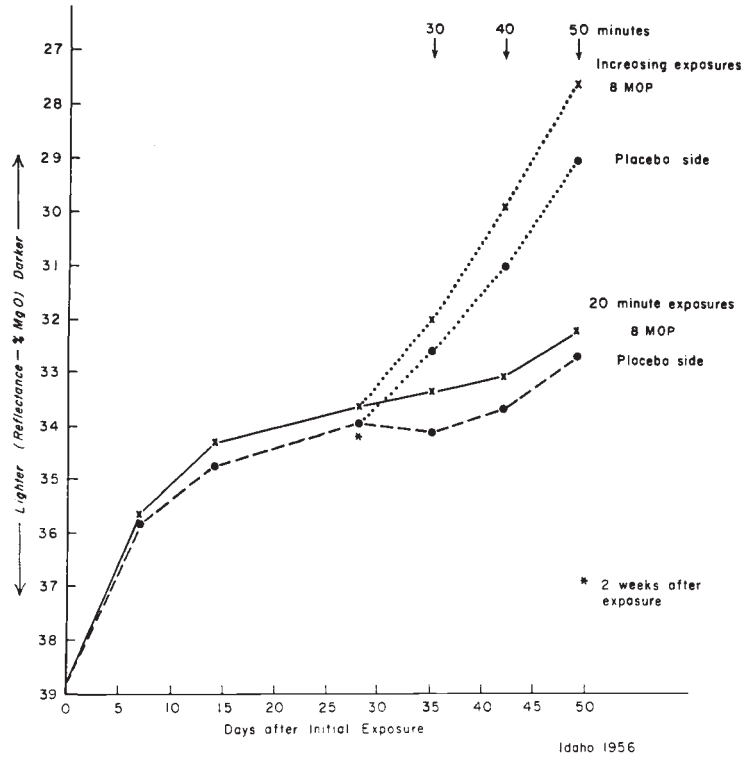

FIG. 6

the 30 men therefore, exceeded 30,000, or about 1,000 different measurements per man.

The course of the mean values for the green reflectance is depicted in Fig. 6. In this graph darkening is represented upward. Throughout the experiment the methoxsalen side was on the average darker than the placebo side. It is evident in the graph that this difference is quantitatively small in comparison to the effects of repeated exposures. Most impressive is the rapid increase in darkening with successive increases in the length of solar exposure.

The green filter gives reflectance values which are affected by both hemoglobin and melanin. The red filter gives chiefly a measurement of melanin (2). The results in the Idaho study for the 40 minute windows are given in Fig. 7. These show the same trends as the previous graph with increase in pigment tending to level off with repetitive equal exposures. With superimposed progressive exposures there is a dramatic and approximately linear increase in skin darkening. This is indicated in the photograph in Fig. 8 (see color plate).

The phenomenon of marked increase in pigment with progressive increase in solar exposure gives us a possible explanation for placebo claims of increased tan. Many of the patients placed on methoxsalen or placebo were also placed for the first time in their lives on a systematic progressively increasing schedule of solar exposures.

In 1956, methoxsalen was administered to 60 male prisoners in Arizona and Idaho State prisons under carefully designed conditions at solar noon to obtain the most consistent sunshine possible from hour to hour and day to day. The tanning effects of 20 mg. of methoxsalen were under these conditions none or quantitatively small. We also had a 58% placebo reaction rate in a double-blind clinical trial (3).

STUDIES AT OREGON DURING THE SUMMER OF 1957

During the summer of 1957, a series of experiments was conducted on the roof of the University of Oregon Medical, School as reported on page 331 of this journal (4).

This experiment demonstrated statistically significant differences in the pigment following

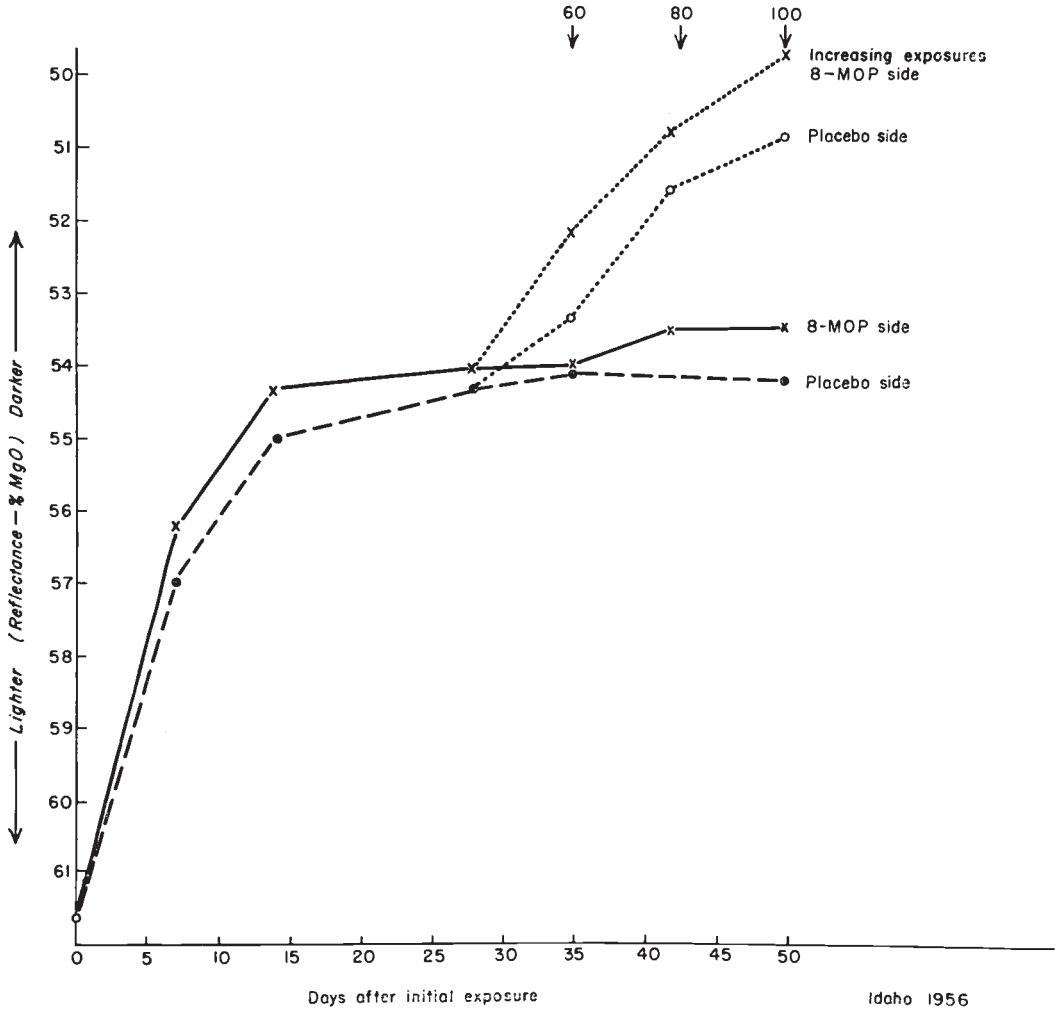

FIG. 7

solar exposure in combination with 30 mg. of methoxsalen as compared to solar exposure without drug. It also demonstrated an approximate doubling in the amount of ultraviolet light required to produce a given level of erythema in the area previously exposed to sunlight and psoralen as compared to the control sides.

SUMMARY

The experiments herein described were designed to test in some detail and with a minimum of experimental error the testimonial and clinical claims that with methoxsalen and exposure to sunlight people "become tanner than ever before."

This series of studies on human subjects used statistically balanced exposures to natural sunlight and doses of methoxsalen ranging from placebo to 40 mg. Areas of the back were exposed and color changes observed and measured with a reflectance meter. In contrast to vivid testimonial evidence, quantitatively large tanning or protective effects could not be demonstrated with 20 mg. of methoxsalen. Conspicuous effects were measured with 30 and 40 milligrams. The experimental conditions were, however, such that it could not be clearly determined whether the greater effects of 30 mg. might be due to differences between Oregon and Idaho sunlight, differences between men and women, or differ-

ences due to relatively small differences in dose, or of drug potency. If the differences are due to dose effects, methoxsalen has a low therapeutic ratio; 20 mg. is barely effective, 30 and 40 mg. are clearly effective in promoting erythema and tanning, while 40, 50, and 75 mg. are clearly dangerous, with approximately one hour of bright sunlight.

The social and psychological importance of a "good tan" is sufficient to contribute to a high placebo action rate (3, 5), which has given a misleading impression of the efficacy of 10 and 20 milligram doses of methoxsalen, which could not be confirmed by quantitative measurements under controlled conditions.

The important role of progressive increment in exposure time in producing tan both with and without methoxsalen was demonstrated.

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